

bridge between the AJs and the actin cytoskeleton. This model was recently challenged and additional properties of acat in regulating the actin cytoskeleton have been described. This new evidence supports the idea that acat function is not limited to cell adhesion. Because these previously unrecognized acat properties were identified in vitro, we chose to characterize acat function in vivo during zebrafish gastrulation to further explore any other novel roles in early vertebrate morphogenesis. a-E-catenin depletion caused defective gastrulation. Surprisingly, acat depleted embryos showed additional defects over e-cadherin (ecdh) depleted embryos. Live cell imaging revealed that depletion of ecdh or acat caused similar defects in cell migration during radial intercalation. However, depletion of acat caused extensive blebbing not observed after ecdh depletion. Double knock down of acat and ecdh abolished the extensive blebbing, indicating that this phenotype is dependent upon acat recruitment to the membrane. Moreover, double knock down of acat and ezrin caused an increase in blebbing. This data demonstrates that acat has actin cytoskeleton regulative properties in vivo and is required for a stable connection between cell membrane and the cell cortex.

doi:[10.1016/j.ydbio.2011.05.131](https://doi.org/10.1016/j.ydbio.2011.05.131)

#### Program/Abstract # 108

##### Mechanisms of primitive streak formation in the mouse embryo

Margot L.K. Williams<sup>a</sup>, Carol Burdsal<sup>b</sup>,  
Ammasi Periasamy<sup>c</sup>, Ann Sutherland<sup>c</sup>

<sup>a</sup>University of Virginia Cell Biology, Charlottesville, VA, USA

<sup>b</sup>Tulane University, New Orleans, LA, USA

<sup>c</sup>University of Virginia, Charlottesville, VA, USA

During gastrulation, amniote embryos acquire the three primordial germ layers – endoderm, ectoderm, and mesoderm – via an epithelial to mesenchymal transition (EMT) that occurs at the primitive streak. Although the primitive streak is vital to the development of all mammals and birds, surprisingly little is known about how it forms and functions. While much has been learned from studies in the chick model system, it is unclear whether these results can be extrapolated to mammalian development. Results from live timelapse imaging and immuno-fluorescent staining studies suggest that the streak does not form by rearrangement of a population of precursor cells, as in the chick, but rather by progressive induction of EMT in the posterior epiblast. Loss of basement membrane is the first step of this EMT, and the only one that coordinates with primitive streak elongation. Matrix metalloproteinases (MMPs) are important for this basement membrane loss, and thus for normal embryonic development. Streak formation is also preceded by widespread weakening of the basement membrane in the embryo's posterior, which does not require MMP activity. This asymmetry represents a previously un-described aspect of early embryo patterning and perhaps a mechanism for streak localization and/or formation. These data contribute significantly to our understanding of how the mammalian primitive streak forms and elongates. Supported by: NIH RO1 HD034807, NIH T32 GM008136.

doi:[10.1016/j.ydbio.2011.05.132](https://doi.org/10.1016/j.ydbio.2011.05.132)

#### Program/Abstract # 109

##### Wnt5b–Ryk pathway provides directional signals to regulate gastrulation movement

Shengda Lin<sup>a</sup>, Lisa Baye<sup>a</sup>, Trudi Westfall<sup>a</sup>, Diane C. Slusarski<sup>b</sup>

<sup>a</sup>University of Iowa, Iowa City, IA, USA

<sup>b</sup>Univ of Iowa Biology, Iowa City, IA, USA

Noncanonical Wnts are largely believed to act as permissive cues for vertebrate cell movement via Frizzled (Fz). In addition to Fz, Wnt ligands are known to regulate neurite outgrowth through an alternative receptor, related to tyrosine kinase (Ryk). However, Wnt–Ryk signaling during embryogenesis is less well characterized. In this study, we report a role for Wnt5b as an instructive cue to regulate gastrulation movements through Ryk. In zebrafish, Ryk deficiency impairs Wnt5b-induced Ca<sup>2+</sup> activity and directional cell movement. Wnt5b–Ryk signaling promotes polarized cell protrusions. Upon Wnt5b stimulation, Fz2, but not Ryk, recruits Dishevelled to the cell membrane, suggesting that Fz2 and Ryk mediate separate pathways. Using co-culture assays to generate directional Wnt5b cues, we demonstrate that Ryk-expressing cells migrate away from the Wnt5b source. We conclude that full-length Ryk conveys Wnt5b signals in a directional manner during gastrulation.

doi:[10.1016/j.ydbio.2011.05.133](https://doi.org/10.1016/j.ydbio.2011.05.133)

#### Program/Abstract # 110

Program/Abstract #110 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

doi:[10.1016/j.ydbio.2011.05.134](https://doi.org/10.1016/j.ydbio.2011.05.134)

#### Program/Abstract # 111

##### Live imaging of cell movement in the developing cochlea confirms periods of convergence and extension

Elizabeth C. Driver<sup>a</sup>, Zoë Mann<sup>b</sup>, Matthew Kelley<sup>b</sup>

<sup>a</sup>NIDCD/NIH Section on Developmental Neuroscience, Bethesda, MD, USA

<sup>b</sup>NIDCD, NIH, Bethesda, MD, USA

The mammalian organ of Corti (OC) consists of a mosaic of four rows of mechanosensory hair cells, surrounded by several types of supporting cells, which extends along the length of the cochlea. The proper formation of this mosaic of cells is critical for auditory function. In early cochlear development, the cells of the prospective OC, identified by expression of p27kip1, are distributed in a domain that is much shorter and broader than is found in the mature OC. As the cochlea grows, the domain of p27kip1-positive cells becomes longer and narrower. This type of cellular rearrangement has been observed in many developmental contexts, and is often achieved via the process of convergent extension (CE). Cellular rearrangements in the cochlear duct are also thought to occur through CE, but the movement of cells within the embryonic cochlear epithelium has never been directly observed. Using cochlear explant cultures and individual fluorescently labeled cells expressing the hair cell gene Atoh1, we have visualized the movements of cells within the developing epithelium in vitro. Time-lapse videos generated over several hours from approximately embryonic days 15 to 16 show movements of cells that are consistent with CE. Moreover, migrating cells exhibit protrusive activity, suggesting that this movement is an active process. Observations of cell movements over several days indicate that most convergent cell migration occurs prior to embryonic day 16, but that the cochlear epithelium continues to extend until at least the equivalent of post-natal day 1. This study is the first to visualize the migration of living cells within the developing cochlea and indicates that active cell movements are necessary for cochlear development.

doi:[10.1016/j.ydbio.2011.05.135](https://doi.org/10.1016/j.ydbio.2011.05.135)